Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 283 (2004) 19–34
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Experimental designed optimisation and stability evaluation of dry suspensions with artemisinin derivatives for paediatric use

M. Gabriëls, J. Plaizier-Vercammen*

Department of Pharmaceutical Technology and Physical Pharmacy, Pharmaceutical Institute, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium

Received 17 July 2003; received in revised form 20 May 2004; accepted 24 May 2004

Available online 23 August 2004

Abstract

There is a great need for oral anti-malaria preparations especially for small children, which are easy to administer and keep their stability under tropical conditions. The purpose of this work was therefore to develop a dry suspension, containing one of the artemisinin derivatives, namely artesunate, artemether and dihydroartemisinin using fast wetting suspending agents, i.e. xanthan gum and Avicel® CL611. For the optimisation of these two variables, namely the suspending agent's content, a Doehlert design was applied. Via preliminary tests on sedimentation behaviour, the limits of both products were determined, respectively 0.1–0.4% (w/v) and 1.0–2.5% (w/v). As responses, sedimentation as a function of time, viscosity and price of the suspension, were evaluated.

The stability tests of the reconstituted suspensions showed bad results for artesunate, even when the pH was adapted. In contrast, dihydroartemisinin showed only 10% degradation within 10 days and artemether was stable at least 21 days. Practically the last one was able to foresee a chemically and physically stable suspension at least during the administration period (5 to 7 days) and was therefore selected for further optimisation concerning taste and appearance. Based on the results of selection tests for the colourant, sweetener and taste masking agent, the following composition was proposed for a suitable dry powder with artemether (AM) as active compound to prepare 100 ml reconstituted suspension: AM 300 mg, Avicel® CL611 2 g, xanthan gum 200 mg, crystalline saccharose 35 g, citric acid monohydrate 150 mg, Nipagine® 80 mg, Nipasol® 20 mg, sodium saccharinate 250 mg, tutti-frutti 250 mg and Sunset yellow 10 mg.

Keywords: Dry powder for paediatric suspension; Artemether; Artesunate; Dihydroartemisinin; Colouring agent; Tast masking agents

1. Introduction

Various derivatives of artemisinin are currently used for the treatment of patients with malaria world-wide. They are increasingly being used, often in combination with other drugs, although our knowledge of the main pharmacological (Navaratnam et al., 2000) and pharmaceutical features (including pharmacokinetics, metabolization, stability, solubility) is still incomplete. Despite of this, new pharmaceutical formulations of an assured quality and long-term stability should be developed but the more, they should be adapted to the needs of the patient (Looareesuwan and Wilairatana, 1998). Especially with malaria, adjusted pharmaceutical formulations for children are indispensable. Most

* Corresponding author. Tel.: +32-2-477-45-92; fax: +32-2-477-47-35.
E-mail addresses: apogama@hotmail.com (M. Gabriëls), jplaizie@vub.ac.be (J. Plaizier-Vercammen).

0378-5173/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2004.05.033

of the oral preparations, containing artemisinin derivatives, are tablets for adult patients. Liquid dosage forms for oral use are the most suitable to administer to small malarious children, who are not able to swallow tablets.

In aqueous solutions, many drugs degrade. And so do the artemisinin derivatives too, especially artesunate (Batty et al., 1996). Moreover, stability of products for tropical countries is a great challenge as these products are exposed to elevated temperatures (up to 40 °C) and relative humidity (up to 90%) (Bos, 1990) especially during transport and storage. Another problem raising with the artemisinin derivatives, is their poor aqueous solubility, as it is for artemether, dihydroartemisinin and artesunate.

A dry suspension can offer several advantages such as maintenance of the chemical stability of the active compounds until reconstitution at the start of treatment. The same suspension can be easily administered to children of different ages by adapting the volume to swallow. The purpose of this work was therefore to evaluate preparation method and the physical stability of the reconstituted suspensions containing artemisinin derivatives, i.e. artesunate, artemether and dihydroartemisinin. For these products, dosage per ml is low, e.g. a loading dose of 3.2 mg/kg body weight per day the first day, and 1.6 mg/kg per day during the following 4 days (Karbwang et al., 1998). The dosage of the active compound in the dry suspension after reconstitution is therefore 3 mg/ml, enabling us to treat small children. Such low dosed suspensions need special preparation requirements: a suspension with almost permanent physical stability. The selection of the best suspending agents is thus required. Based on formula of FMC Europe (Brussels, Belgium) (internal communication), a combination of xanthan gum and Avicel® CL611 seemed to be the suitable one for dry suspensions.

Xanthan gum is a high molecular weight anionic polysaccharide produced from microbial fermentation (Ofner et al., 1989), that functions as a suspending agent (Bhargava and Nicolai, 1989). It is soluble in water and imparts its high viscosity at low concentration with thixotropic flow characteristics, which increase with increasing concentration. Xanthan gum is particularly useful as a suspending agent because of this characteristic. Almost no changes in viscosity are noticed, due to increasing temperature, which is an interesting characteristic for suspensions, used in tropical conditions. Solutions of xanthan gum are compatible with many adjuvants such as sugars and preservatives, if they are not cations. However, the solutions are susceptible to microbiological degradation on prolonged storage and do require a preservative to maintain the microbial integrity of the product (Bhargava and Nicolai, 1989).

Avicel® CL611, containing microcrystalline cellulose 85% (w/w) and carboxymethyl-cellulose 15% (w/w), can be added to enhance the thixotropic characteristics of the reconstituted suspension, and the flow abilities of the dry powder.

All other excipients, the preservatives and the ones, which influence the aesthetic aspect of the suspension, were selected in the first place for their suitability in children.

Sweeteners, such as sodium saccharinate, and flavours are extremely important to make the preparation attractive for paediatric patients. Colourants are intended to provide a more aesthetic appearance to the final suspension. Common water-soluble agents, used in suspensions are quinoleine yellow, erythrosine and Sunset yellow. Their concentration ranges were selected, based on data from literature (BASF, 1997) and registered pharmaceutical formulations. They were firstly controlled on their suitability for small children.

The purpose of this work in the first place is thus to develop a dry suspension powder from which a ‘permanent’ stable suspension can be prepared after reconstitution. The excipients should be kept as low as possible, especially the expensive ones, such as the suspending agents. Therefore, an optimisation technique will be applied. In a second step, all attention is focused on the selection of the taste masker and the colourant(s). Their attraction to adult and paediatric patients and their behaviour in the reconstituted suspension are also investigated.

2. Material and methods

2.1. Material

The active substances, artesunate, artemether and dihydroartemisinin are all purchased from Arenco

Pharmaceutica (Geel, Belgium). The excipients, used in the preparation of the suspension, were the following: xanthan gum Rheogel® from CNI (Neuilly-sur-Seine, France), Avicel® CL611 from FMC Europe (Brussels, Belgium), citric acid monohydrate from Merck (Darmstadt, Germany) and methyl parahydroxybenzoate (Nipagine®) and propyl parahydroxybenzoate (Nipasol®), respectively from Federa (Brussels, Belgium) and Flandria (Ghent, Belgium) and microcrystalline saccharose from the Tiense Suikerraffinaderij (Tienen, Belgium).

Addition of the following excipients in the basic suspension was also investigated: the taste masking agents, blood orange and tutti-frutti from Haarmann and Reimer GmbH (Holzminden, Germany); the sweeteners sodium saccharinate from Alpha Pharma (Zwevegem, Belgium) and sodium cyclamate from Federa; as colourants quinoleine yellow (E104) from P. Entrop (Mechelen, Belgium), erythrosine 85% g/g (E127) and Sunset yellow (orange yellow; E110) from Federa were used. For the preparation of standards and dry suspension samples, alcohol 94% (v/v) was used. For the derivatization of the spots in the TLC-method, 4-methoxybenzaldehyde, sulfuric acid 95–97% (v/v) and acetic acid 96–98% (v/v) from Merck were utilized. Aqueous solutions were all prepared with MQ-water, even the reconstitution of the suspension for investigation.

2.2. Preparation of the dry suspension

Investigation was performed on four different suspensions, namely one without active substance, the "blank" suspension, and three containing respectively artesunate, artemether and dihydroartemisinin.

For a dry suspension in which xanthan gum and Avicel® CL611 were employed as suspending agents, the following composition was selected for further research to obtain a permanent physically stable suspension: active substance 0 mg (for the blank) or 300 mg, crystalline saccharose 35 g, Avicel® CL611 q.s., citric acid monohydrate 0.09 g, xanthan gum (Rheogel®) q.s., Nipagine® 0.08 g, Nipasol® 0.02 g, water up to 100 ml (="basic" suspension).

The following procedure was applied to prepare the dry suspension. The smallest amount of powder was mixed with the same amount of another excipient, following the principle of the geometric dilution. The powder was then extra mixed in a Turbula mixer from Willy A. Bachofen (Basel, Switzerland) during 10 min.

To prepare the reconstituted suspension, an appropriate volume of water was added to the dry powder in two steps (circa 70 ml up to a total volume of 100 ml). A first content of ±35 ml was added and the suspension was shaken by hand until a homogeneous product was obtained. Another ±35 ml of water was added and shaken up to homogeneity.

2.3. Determination of the rheological and sedimentation characteristics of the reconstituted suspension

2.3.1. Rheology

The rheologic parameters of the prepared suspensions, in terms of viscosity, are determined by use of the steady shear method, measuring the "non-Newtonian viscosity". It is therefore essential that the instrumentation used, is able to operate at a variety of rates of shear (Martin et al., 1993a).

Rheology of all suspensions of the design were performed with a Carrimed viscometer from Paramus (New York, USA). All measurements were performed after eliminating all thixotropy from the suspension. From these results, the viscosity (η) (Pa s) was calculated from a shear stress (τ) (Pa) at a shear rate (D) of $0.05\,s^{-1}$, using the formula $\eta = \tau/D$.

The limits on viscosity were selected as such that the suspension reaches a physically stable state. Therefore, the sedimentation characteristics were also evaluated.

2.3.2. Sedimentation characteristics

To study of sedimentation in our suspensions, the sedimentation volume was determined as a function of time.

The sedimentation volume F is defined as the ratio of the final, equilibrium volume of the sediment, V_u to the total volume V_0 before settling, as expressed in the following equation: $F = [V_u/V_0]$ (Martin et al., 1993c).

In this study, the sedimentation volume was determined as a function of time. 50.0 ml suspension (height = 9 cm) was decanted in a cylinder of 100 ml with a diameter of 2.5 cm. After 1 h and 1 week, the sedimentation volume F was determined.

2.4. Selection of the best dry suspension formulation, using an experimental Doehlert design

In a first set of experiments, the concentration ranges of both suspending agents, xanthan gum and Avicel® CL611 were determined. For further optimisation of the composition of the dry powder in terms of suitable viscosity and sedimentation, a 2^2 factorial Doehlert design, as described by Hu and Massart (1989), was applied. Practically, the effect of two factors, namely the concentration of both suspending agents, Avicel® CL611 and xanthan gum, was studied. All other excipients in the formula were kept constant. The Doehlert design with two factors consists of a hexagon, containing six corner points, all having the same distance to the central point. For each corner point, an appropriate suspension was prepared. The central point is prepared two times, to make an estimation of the error. Practically, eight powder mixtures were made.

Table 1 shows the level of variables, coded values and content of each point of the design.

In Fig. 1B, the position of the different points (suspensions D_1 up to D_8), is presented in the Doehlert design.

Since each variable was not expressed in the same unit and level, it is difficult to define the extent of the variable's influence on the response. So, using coded values normalized them. The general form of the quadratic model, which describes the effect of the variables, is presented in the following formula:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2 \quad (1)$$

In which X_1 and X_2 are, respectively, the concentration of xanthan gum and Avicel® CL611, both in coded values, and X_1X_2 is the interaction term between them. With aid of multiple linear regression, the values of the model coefficients (b_i) were calculated. The response is the viscosity and the sedimentation behaviour as a function of time, in terms of the sedimentation volume F. The procedure for the determination of the responses is explained in Section 2.3.

2.5. Investigation of the chemical stability and the crystal growth of the active compound(s) of the reconstituted suspensions

2.5.1. Investigation of the chemical stability of the active compounds

Samples were taken at different time intervals of storage, in the beginning every 2 or 3 days, depending on the samples' stability, and evaluated for their chemical stability. Practically, the suspension powder, containing the amount of suspending agents, as selected from the design, and other excipients as presented in the introduction, was prepared and divided in 10 bottles, in which 25 g reconstituted suspension can be prepared. 75 mg active compound was manually added to each bottle. At different time periods, a sample was reconstituted with MQ water. All samples were analysed with TLC. The frequency of further analysis depends on the chemical stability of the preparation. A carefully weighed amount of suspension (±2.5 g) was dissolved in alcohol and then diluted up to an appropriate volume, depending of the degradation level of the active compound. The upper phase was spotted on the TLC plate. Standard solutions of artesunate or artemether, having a concentration between 20 and 80 mg/100 ml, were prepared in alcohol. Standards and samples are all analysed with thin layer chromatography/densitometry, as mentioned in Section 2.7.1.

Table 1
Level of variables, coded values and concentration of each point of the Doehlert design (1 to 6: corner points, 7–8: central point)

	Sample no.							
	1	2	3	4	5	6	7	8
[Rheogel®] % (g/v)	0.175	0.325	0.400	0.325	0.175	0.100	0.250	0.250
Coded value	−0.5	0.5	1	0.5	−0.5	−1	0	0
[Avicel® CL611] % (g/v)	2.500	2.500	1.750	1.000	1.000	1.750	1.750	1.750
Coded value	0.866	0.866	0	−0.866	−0.866	0	0	0

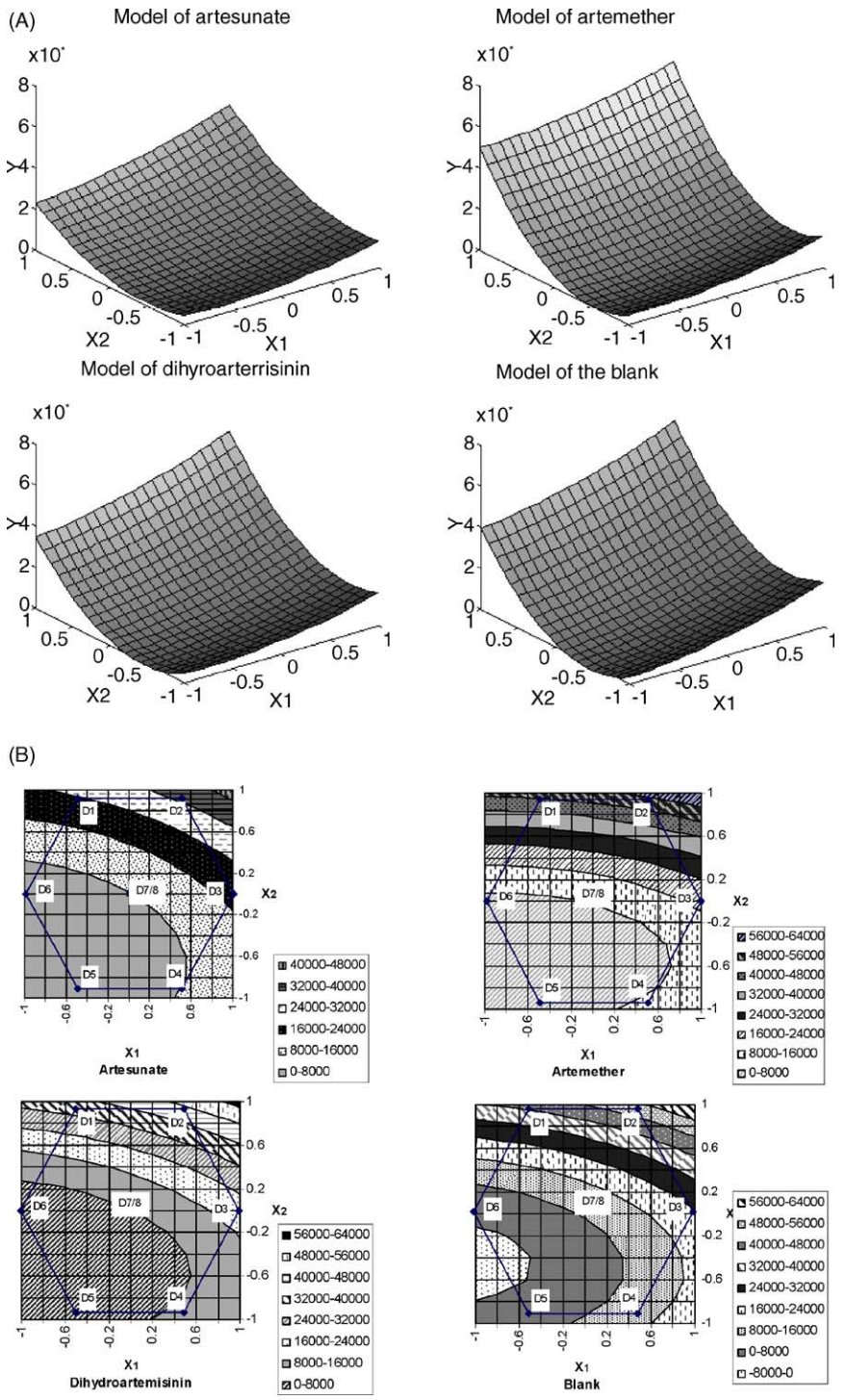

Fig. 1. 3D and 2D presentation of the model, describing the viscosity responses of each suspension (X_1 = concentration of xanthan gum in %w/w; X_2 = concentration of Avicel® CL611 in %w/w; Y = viscosity (m Pa s) as response) (at $D = 0.05\,s^{-1}$).

2.5.2. Crystal growth of artemether in the reconstituted suspension

The influence of the temperature (25 and 45 °C), time of storage (1 week up to 4 weeks) and packaging (plastic or glass) on the artemether in the reconstituted suspension was evaluated by control on its crystal growth. Therefore, the length and the width of the crystals was measured with a microscope with lens from Nikon (Tokyo, Japan) and an ocular of Carl Zeiss (Rodemark, Germany) (magnitude 10 × 10; scale division: 15 μm). The significance of change in crystal size was evaluated by performing a two-way ANOVA test at level 5% ($\alpha = 0.05$; if experimental value $P > 0.05$: not significant). Per set of samples, the length and width was measured on 10 crystals.

2.6. Evaluation of the design

As already mentioned before, a quadratic model is calculated for each response, if valid.

Herewith, the relation between the independent variables (the experimental ones) and the dependent ones is also investigated. The quality of a model is evaluated by using regression analysis or ANOVA and the QC.

2.6.1. ANOVA analysis of the model

In the regression analysis or ANOVA-test of a model, the coefficients of the equation, consisting of five terms, were calculated. If the coefficients' variance $P > 0.05$, one could conclude that the corresponding term has no significant value within the equation (Massart et al., 1989). Practically, the corresponding variable is estimated to have a reduced or no influence on the measured response.

2.6.2. Quality-coefficient (QC)

The quality coefficient is a parameter checking the general quality of the calculated model via determination of the standard deviation between the experimental and the calculated results.

This coefficient is calculated by using the following formula (Vankeerbergen et al., 1996):

$$\mathrm{QC}\,(\%) = \sqrt{\frac{\sum_{i=1}^{n}((y_i - \hat{y}_i)/\bar{y})^2}{n-1}} \times 100$$

where n represents the number of data points, y_i is the response measured, $\hat{y}_i$ is the response predicted by the model and $\bar{y}$ is the mean of the measured responses.

2.7. Analytical methods

2.7.1. TLC and densitometry determination

To control the stability of the active compound in the reconstituted suspension as a function of time, the preparations, stored at 45 °C, were evaluated via a TLC densitometric method.

The analysis was performed on RP-C18 F254S TLC plates (10 cm × 20 cm), having pre-coated layer thickness 0.25 mm from Merck. Plates were spotted with 10 μl aliquots of the standard or sample solutions with a Linomat IV from Camag (Lot, Belgium) under a continuous drying nitrogen stream in a 1 cm band at 2 cm from the bottom on the TLC plate.

The development was performed in a developing chamber tank from Camag, saturated overnight at 25 °C with a mobile phase, composed of acetonitrile/water (50/20 v/v).

The spots were visualised by dipping the thin layer plate in dipping chamber (20 cm × 20 cm) from Camag, containing a derivatization solution, which consists of 2 ml 4-methoxybenzaldehyde, 4 ml sulphuric acid 95–97% and 20 ml acetic acid 98%, dissolved in 100 ml alcohol 94% and 80 ml water. Then, the plates were activated at 110 °C during 10 min, utilizing a plate heater from Camag. Quantitative densitometric determination was performed with a Zeiss-Densitometer (Oberkochen, Germany) at 565 nm. The scanning of the plates was done by measuring 200 points over a vertical distance of 5 cm during 1 min. Densitograms were generated from transmission measurements of the samples and the blank. The respective absorbance values were calculated and peak areas were quantified with aid of the Peakfit program from Jandel (Erkrath, Germany).

2.7.2. Visible spectrophotometry determination

To control the eventual adsorbance of colourants onto other excipients in the resonstituted suspension, aqueous solutions of these substances were measured with visible spectrophotometry at their appropriate

wavelength. Therefore, an UVIKON 860 spectrophotometer from Kontron Instruments (Zürich, Switzerland) was used.

2.7.3. Separation of the colourants

To separate the colourant from the reconstituted suspension, a filtration and centrifugation technique was applied, investigating different filters and using different centrifuges.

The following materials and instruments were employed: paper filters (Ø 125 mm) of Schleicher & Schuell (Dassen, Germany) and Whatman sterile filters (Ø 25 mm) from Whatman (Maidstone, Great-Britain); Mistral 400 MSE centrifuge refrigerated from Beun De Ronde (La Abcoude, Belgium) and Sorvall RC-5B centrifuge refrigerated susperspeed from Du Pont Instruments (Herts, Great-Britain) with a SS 34 rotor from Sorvall Instruments (Redwood City, USA).

Different conditions of centrifugation (duration: 5 to 20 min; and velocity: 1000 rpm (175 × *g*) up to 12,000 rpm (2260 × *g*)) were tested.

3. Results and discussion

In the first part of our study, the concentration ranges for the different agents in the suspension were explored. In the second part, the concentration of each suspending agent was considered as the varying parameters of the Doehlert design. As responses, the viscosity and sedimentation behaviour was considered. With aid of a multiple linear regression program in Microsoft Excel, the equation of the model, which expresses the influence of the variables on each response, was calculated.

3.1. Pretests for the determination of the concentration ranges for both suspending agents

It is known from literature that the concentration of suspending agents depends strongly on the desired characteristics of the suspension, which should be prepared.

Normal concentrations for xanthan gum, if used alone, ranged to 0.5% (g/v) (Plaizier-Vercammen, 1991); for Avicel® CL611 up to 3% (g/v) is described. Therefore, some blank suspensions, as presented in Table 2 were prepared, containing xanthan gum between 0 and 0.5% (g/v) and Avicel® CL611 from 0 to 3% (g/v). They were investigated on their sedimentation volume *F* following the method described in Section 2.3. Results are also presented in Table 2.

Table 2
Composition and sedimentation behaviour of reconstituted suspensions for the determination of the concentration ranges of suspending agents, xanthan gum (Rheogel®) and Avicel® CL611

Sample	[Rheogel®] % (g/v)	[Avicel® CL611] % (g/v)	$F_{1\,h}$	$F_{1\,week}$
1	0.000	1.5	0.11	0.11
2	0.125	3.0	1.0	0.98
3	0.375	0.0	1.0	1.0
4	0.500	1.5	1.0	1.0

To obtain an acceptable suspension, *F* should be at least 0.9 for 1 h but a longer period is preferred for our purpose. For the first suspension, containing only Avicel® CL611 as suspending agent, first signs of sedimentation were already noticed within 10 min. After 1 h the value *F* is 0.11, which means that the suspension almost totally deposited on the bottom of the cylinder. The second suspension shows only a slight sedimentation after 1 week ($F = 0.98$). The other ones are already permanently stable but they have a visually high viscosity. The determination of the ranges of the concentrations of the suspending agents is therefore based on suspension 2 to 4. From these results we can suggest that the viscosity of the suspension is mostly determined by the concentration of xanthan gum.

Based on these results, the following concentration ranges were selected, as follows:

Xanthan gum (Rheogel®)	0.1 to 0.4% (g/v)
Avicel® CL611	1.0 to 2.5% (g/v)

3.2. Application of the Doehlert design for the optimisation of the dry suspension content

Based on the selected ranges of the two variables (Section 3.1), the different dry suspension samples were equally distributed following the principles of the Doehlert design. Their composition is given in Table 1.

3.2.1. Investigation of the sedimentation behaviour and the viscosity of the different reconstituted suspensions composed according to the Doehlert design

One sample of each design point was investigated for viscosity with the Carrimed viscometer.

3.2.1.1. Sedimentation behaviour. It is clear that the suspension with the lowest amounts of suspending agents seemed to develop a fast sedimentation; especially suspension 6 containing only 0.1% xanthan gum, forms a sediment quite fast ($F = 0.34$ after 4 days and $F = 0.11$ after 1 week). Slight enhancement of the xanthan gum changes the sedimentation behaviour drastically. Suspension 5, containing 0.175% xanthan gum and additionally a lower content of Avicel® CL611 than suspension 6 (1.0 and 1.75%, respectively), showed only a slight sedimentation after 4 days and a F value of 0.1 after 1 month. It is obvious that xanthan gum plays the most important role in the sedimentation behaviour and thus the physical stability of the suspension. All other suspension were stable for at least 1 week and, with exception of suspensions 3 and 4, for a whole month.

3.2.1.2. Measurements with the Carrimed viscometer. Rheograms were determined with the Carrined viscometer, from each suspension containing one of the investigated active compounds, artesunate, artemether and dihydroartemisinin, and from the "blank" suspension. Based on the measurement of the rheogram, the viscosity was determined at a shear rate of $0.05\,s^{-1}$.

Each suspension was measured three times and the mean viscosity was evaluated (Table 3).

Also from these results, we can observe that the viscosity is mostly influenced by the xanthan gum compared to the Avicel® CL611 concentration. Based on the results of the sedimentation behaviour, we could conclude that the viscosity should be at least 8000 m Pa s. Suspensions having higher values can fulfil the physical stability, required during the administration period (1 week).

When considering the viscosity values for the suspensions with different active compounds, the results are similar. The viscosity values for suspensions 2 and 3 are consistently in the range of 20,000 m Pa s or higher. The other suspensions show more comparable results.

3.2.2. Determination of the best model of the Doehlert design

To estimate the dry suspension composition for our purposes, which will be selected in terms of the concentration of both suspensding agents, xanthan gum (Rheogel®) and Avicel® CL611, the best model for the Doehlert design was investigated.

Therefore, the significance of a term in the polynomial equation via calculation of the P value (see Section 2.6) and the QC was determined for each suspension.

For the four series of suspensions, the model was calculated (Table 4).

For each model calculated for the response of viscosity, the QC% is always smaller than 5% and the regression coefficient is always higher than 0.9, indicating that the model describes quite correctly the experimental results. The best results are obtained for artemether, having the lowest QC%, then dihydroartemisinin. For all suspensions with active

Table 3
Viscosity η for all reconstituted suspensions at a shear rate of $0.05\,s^{-1}$, based on measurements with the Carrimed viscometer

Suspension	Xanthan gum (g/g%)	Avicel® CL611 (g/g%)	AS	AM	DHA	Blank
1	0.175	2.500	5736	12124	6895	5938
2	0.325	2.500	31360	49513	43177	47473
3	0.400	1.750	18123	18007	20168	23108
4	0.325	1.000	7756	7822	8849	12383
5	0.175	1.000	3173	2807	2770	2976
6	0.100	1.750	3931	5972	2476	1639
7	0.250	1.750	8339	8630	7807	7408
8	0.250	1.750	7147	8561	7748	9854

AS: artesunate; AM: artemether; DHA: dihydroartemisinin.

Table 4
Quality and coefficients (b_i) and their P value of the calculated model for each series of suspensions

Model	b_0	b_1X_1	b_2X_2	$b_{11}X_1^2$	$b_{22}X_2^2$	$b_{12}X_1X_2$	QC (%)	R^2
Artesunate								
b_i	7743	7096	12177	3284	9928	2902	2.51	0.999
P	0.029	0.023	8.0×10^{-4}	0.031	0.036	0.052		
Artemether								
b_i	8596	6017	23492	3394	21621	1157	0.10	0.999
P	8.0×10^{-6}	1.1×10^{-5}	7.2×10^{-7}	1.0×10^{-4}	2.5×10^{-6}	2.0×10^{-3}		
Dihydroartemisinin								
b_i	7778	8846	18222	3545	17236	3195	0.81	0.999
P	7.2×10^{-6}	3.7×10^{-6}	8.7×10^{-7}	6.9×10^{-5}	2.9×10^{-6}	2.0×10^{-3}		
Blank								
b_i	8631	10734	19493	3743	19993	1533	3.73	0.998
P	0.9×10^{-3}	4.3×10^{-3}	1.3×10^{-3}	9.2×10^{-2}	3.7×10^{-3}	0.39		

compounds, a P value <0.05 was observed for the X_1X_2-term. This suggest that there is an interaction between these excipients.

3.2.3. Selection of the most suitable powder composition for a dry suspension from the Doehlert design results

From previous viscosity tests, we noticed that at least 8000 m Pa s is required to obtain a physical stable suspension (with a F value of 1 over a longer period). All reconstituted suspensions of the design showed a good physical stability or a good redispersibility and no caking occurred after storage over 1 month. Based on the sedimentation behaviour, two suspensions could already excluded namely no. 5 and 6, having lowest concentrations of xanthan gum, combined with low concentration of Avicel® CL611. Based on the viscosity, the suspensions no. 4, 7 and 8 seemed to be the most suitable ones, as they show viscosity values of respectively 12,000, 7408 and 9854 m Pa s at $D = 0.05\,s^{-1}$. Additionally they are physically stable as required for our purpose. Based on the 3D- and contour-plots, given in Fig. 1, the zones in which the best suspension composition should be selected, could be determined. Additionally to their viscosity and sedimentation characteristics, another aspect should be taken into account. As the dry powder for suspension is destinated for tropical countries, the price of the excipients in the formulation should be considered. Based on the prices of the suspending agents (xanthan gum 7.91 Euro/kg and Avicel® CL611 3.84 Euro/kg), the content of xanthan gum should be kept as low as possible.

The zone including all suspensions with the suspensions having the minimal concentrations of the suspending agents is described as follows:

Artesunate	
(−0.8; 0.25)	0.130% (w/v) xanthan gum and 1.966% (w/v) Avicel® CL611
(0; 0)	0.250% (w/v) xanthan gum and 1.750% (w/v) Avicel® CL611
(0.5; −0.866)	0.325% (w/v) xanthan gum and 1.000% (w/v) Avicel® CL611

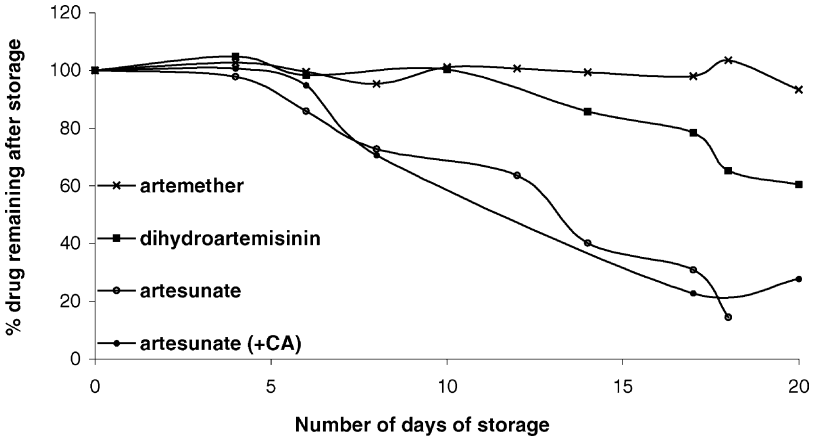

Fig. 2. Degradation of artesunate, artemether and dihydroartemisinin as a function of time (CA = citric acid).

Artemether	
(−0.9; 0.05)	0.115% (w/v) xanthan gum and 1.793% (w/v) Avicel® CL611
(0; 0)	0.250% (w/v) xanthan gum and 1.750% (w/v) Avicel® CL611
(0.7; −0.6)	0.355% (w/v) xanthan gum and 1.230% (w/v) Avicel® CL611
Dihydroartemisinin	
(−0.9; 0.25)	0.115% (w/v) xanthan gum and 1.966% (w/v) Avicel® CL611
(0; 0)	0.250% (w/v) xanthan gum and 1.750% (w/v) Avicel® CL611
(0.55; −0.6)	0.332% (w/v) xanthan gum and 1.230% (w/v) Avicel® CL611
(0.3; −0.866)	0.295% (w/v) xanthan gum and 1.000% (w/v) Avicel® CL611
Blank	
(−0.9; 0.15)	0.115% (w/v) xanthan gum and 1.880% (w/v) Avicel® CL611
(−0.5; 0.4)	0.175% (w/v) xanthan gum and 2.096% (w/v) Avicel® CL611
(−0.7; −0.7)	0.145% (w/v) xanthan gum and 1.144% (w/v) Avicel® CL611

For each of the suspensions, the one with the lowest amount of xanthan gum, still having a suitable viscosity, was selected: 0.20% w/v xanthan gum and 2.00% w/v Avicel® CL611 for artesunate; 0.20% w/v xanthan gum and 2.4% w/v Avicel® CL611 for artemether; 0.20% w/v xanthan gum and 2.30% w/v Avicel® CL611 for dihydroartemisinin and 0.2% w/v xanthan gum and 2.00% w/v Avicel® CL611 for the blank.

The selection of a dry powder for suspension, the "blank", without active compound, can be of interest as excipient for other low-dose preparations.

3.3. Preliminary stability test on the selected dry suspensions of artesunate, artemether and dihydroartemisinin after reconstitution

As some of the investigated compounds are known to be sensitive to chemical instabilities, especially artesunate (Batty et al., 1996), a stability test was performed with the reconstituted suspension for the whole period of administration, which is at least 5 days, but a longer period is preferable. Practically, stability was followed over a period of 3 weeks. Fig. 2 represents the chemical stability results of artesunate with a small and a high content of citric acid, artemether and dihydroartemisinin in the reconstituted suspension. The normal pH of the suspension is in the range

of 4–4.5. By addition of citric acid the, pH reaches 3–3.5.

The degradation curves of the actives in the reconstituted suspensions (Fig. 2) at 25 °C, suggest a 'lag'-time of 5 days for artesunate and 10 days for dihydroartemisinin. After that period, the actives are degrading. The results suggest a zero order kinetic, as expected in suspensions (Martin et al., 1993b). Artesunate degrades up to 50% within 12 days, probably due to enhanced solubility of artesunate in the solvent of the suspension, compared to the other actives. In a second test (Fig. 2), higher amount of citric acid, namely 1 g per 100 g suspension powder, was added, avoiding artesunate to dissolve partially, but did not improve the stability of the active.

Dihydroartemisinin showed after the lag-time degradation up to 65% after 21 days. Stability during the 7 days of treatment was proven, however too uncertain to be used in tropical countries, in which higher temperatures and poor storage conditions can be expected. On the contrary, artemether remains stable during the whole investigated period, namely 21 days.

The crystal growth of artemether was evaluated in the reconstituted suspension in presence of all finally selected excipients, kept at different temperatures, in different packaging and during different storage periods. From the measurements of the crystal size after 1 week, values of length and width of the crystals ($n = 10$) per two samples of the same set were respectively (155 and 1007 μm) and (31 and 294 μm). No significant differences after 1 week were noticed.

But after 4 weeks, significant crystal growth was observed for 25 and 45 °C ($P = 0.024$) and after 4 weeks compared to 1 week ($P < 0.0005$).

The packaging did not show any influence on the growth of the artemether crystals.

Therefore, we could conclude that the temperature and conservation period has to be clearly defined in the storage conditions of the reconstituted suspension.

3.4. Investigation on other ingredients in the dry suspension with artemether

As the presented formulation is indicated for malarious children, much attention was paid to this not unimportant aspect of colour and flavour in the processing of the dry suspension, when developing pharmaceuticals for paediatric or geriatric populations (Worthington, 2000). Both groups have difficulties to take the traditional solid dosage forms and children respond dramatically to flavour, refusing to take a distasteful medicine even with the promise that it will make them feel better.

Two taste masking agents, available as a powder and accepted by the Pharmacopoeia, were tried, namely tutti-frutti and blood orange. An extra sweetener, additionally to the saccharose, was added. Aspartame and sodium saccharinate were therefore evaluated. Both agents are required to mask the bitter taste of artemether. Colourants are intended to provide a more aesthetic appearance to the final suspension. Water-soluble colourants often used are quinoleine yellow, erythrosine and Sunset yellow. The preferred colour for our suspension is orange. Therefore, either a mixture of quinoleine yellow (yellow colourant) and erythrosine (red colourant) or pure Sunset yellow (orange colourant) can be used.

3.4.1. Optimising the flavour quality of the suspension

In the optimisation of the aesthetic aspect of our formulation, a first set of experiments was focused on optimising the taste, which was evaluated at two levels to select the sweetener and the flavour. Taste tests on both were performed with students, aging around 21 years. The following sweeteners were presented in the test: saccharose which was taken as reference for the sweetness, having a relative sweetness (R.S.) of 1, cyclamate (R.S. 15–30) and sodium saccharinate (R.S. 300–500) (Harwood et al., 1989). Another common sweetener, aspartame (R.S. 200), was abandoned as it is dissuaded for children under 3 years, which can limit the use of the suspension for small children. The flavours, blood orange and tutti-frutti, combined with sodium saccharinate, were tested in children and adults from three different African countries, Tanzania, Kenya and Uganda (Table 5).

The group of student volunteers (7 males and 14 females) firstly tested solutions of sodium saccharinate (46 mg/20 ml) and sodium cyclamate (10 mg/20 ml) in water. They preferred sodium saccharinate due to its more neutral aftertaste compared to the cyclamate. Later on suspensions, containing all exciepients (xanthane gum, Avicel® CL611, sugar, the preservatives, sodium sacharinate as sweetener, and artemether as

Table 5
Flavour preference results from children and adults of three African countries

	Orange	Tutti-frutti
Kenya		
Adults	3	17
Children 5–12 years	5	72
Uganda		
Adults	13	13
Children 5–12 years	8	24
Tanzania		
Children 5–12 years	14	10
Total	43 (24%)	136 (76%)

active compound with the fruit tastes were evaluated. Blood orange 30 mg and tutti-frutti 50 mg per 10 ml reconstituted suspension were added. Both flavours are accepted similarly by this age group; most of them perceived a sweet aftertaste of the tutti-frutti. From the results from tests in African patients (Table 5), we can conclude that the tutti-frutti was overall preferred by the children; even adults desired the fruit flavour. At least 76% of the patients selected it. Only in Tanzania, a preference for orange was observed, which can be explained by the habitual mass consumption of oranges in the country. The fact that 76% chose tutti-frutti flavour indicates the superiority of tutti-frutti over orange flavour.

3.4.2. Optimising the visual aspect of the suspension

To optimise the visual aspect of the suspension, an orange colour in combination with a fruit taste was selected. Much attention is paid to that as it can enhance the patient's compliance, when treated with this product. Pharmaceutical acceptable colourants to obtain this colour are, a combination of erythrosine and quinoleine yellow or Sunset yellow alone.

These colours were tested on their suitability in the reconstituted suspension, colour stability at different temperatures, and possible adsorbance on the excipients and packaging.

3.4.2.1. Analytical aspects of the colours. In a first set of experiments the analytical aspects of the colours were investigated via colorimetry. Therefore, the maximal wavelength and range of linearity via a calibration line was determined. A spectrum of each of the colours is presented in Fig. 3.

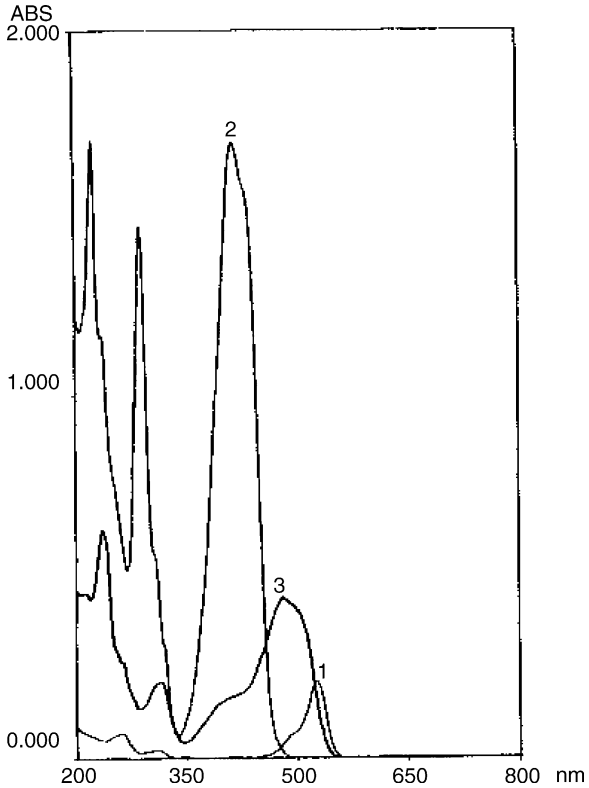

Fig. 3. Spectrum of the colours in aqueous solutions: (1) erythrosine 0.25 mg/100 ml (λ_{max}: 526 nm); (2) quinoleine yellow 3.5 mg/100 ml (λ_{max}: 415 nm); (3) Sunset yellow 1 mg/100 ml (λ_{max}: 479 nm).

The following results were obtained for aqueous solutions of erythrosine, quinoleine yellow and Sunset yellow. Their maximal wavelength in the visible range was respectively 526, 418 and 479 nm.

Linearity was obtained in the range of 0 to 2 mg/100 ml for erythrosine and in the range of 0 to 4 mg/100 ml for the both yellow colours. The equations for their calibration lines and their respective correlation are the following:

- Erythrosine: $y = 0.9074x + 0.0013$ ($r = 0.9999$)
- Quinoleine yellow: $y = 0.4721x + 0.0054$ ($r = 0.9998$)
- Sunset yellow: $y = 0.4589x - 0.0125$ ($r = 0.998$)

where y is the absorbance at maximal wavelength and x is the concentration of the analyte expressed as mg/100 ml.

As a combination of erythrosine and quinoleine yellow will be applied to obtain an orange colour, both colours will be measured in the same solution. At their λ_{max} they do not show any overlap for the investigated concentration and each colour can be quantified at its own maximal wavelength.

3.4.2.2. Determination of the colour concentrations in the reconstituted suspension to obtain an orange colour. Series of aqueous solutions were prepared from erythrosine and quinoleine yellow in the range of 1:5 to 1:50 (g/g). The ratio with the most suitable colour was 1:25 (g/g). It was further investigated in which amount the colours should be added. The highest amount of each colouring agent added to 100 ml reconstituted suspension, is 0.8 mg erythrosine and 20 mg quinoleine yellow per 100 ml, which are pharmaceutical acceptable concentrations. With this ratio, different concentrations were investigated to select the minimal amount required for a suitable colour in the suspension. Another alternative is Sunset yellow, from which the orange colour was immediately obtained when 0.01 g/100 ml was added to the reconstituted suspension.

3.4.2.3. Adsorption of the colourants to containers. In a first set of experiments, the adsorption on the packaging was evaluated. Aqueous solutions were stored in plastic and glass bottles at 25 and 45 °C. Every week, a sample was taken and subjected to colorimetry. The absorbance was measured for each colour at maximal wavelength. Results are presented in Fig. 4.

For erythrosine, adsorption was suggested already after 1 week, especially in plastic bottles, as for each storage condition a loss of at least 10% after 1 week was noticed. For quinoleine yellow, no adsorption was observed. For Sunset yellow, some changes were noticed in the last week of measurement, namely after 3 weeks of storage in glass at 45 °C possibly due to absorption. However, it is stable for the period of administration of the suspension to the patient. Only based on these results, our preference goes to the Sunset yellow colour, but further investigation is required for definitive selection.

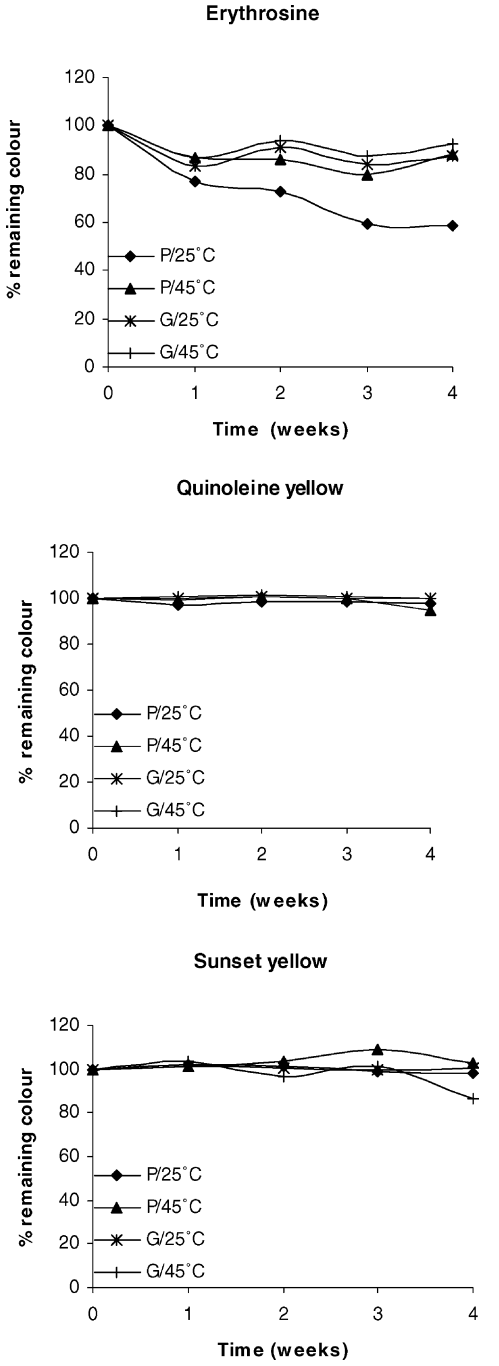

Fig. 4. Determination of the adsorption of the colours onto the packaging as a function of time at different temperatures (G = glass; P = plastics).

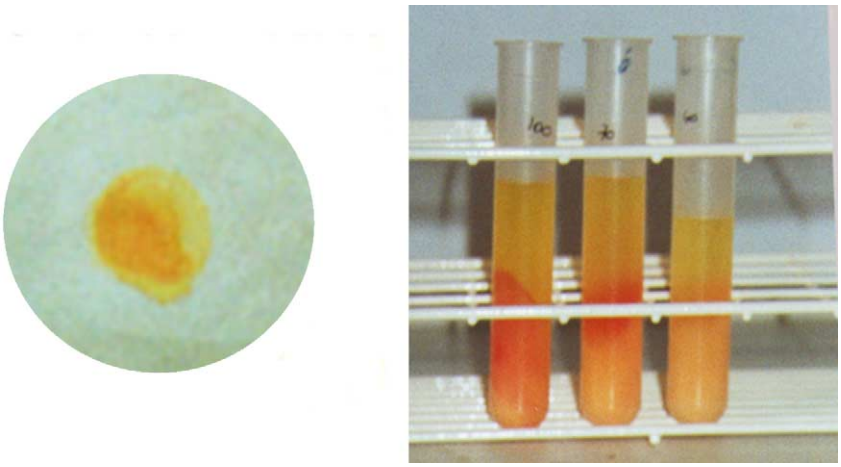

Fig. 5. Adsorption of erythrosine and quinoleine yellow on non-water-soluble excipients after filtration (left) and erythrosine on the excipients after centrifugation of the reconstituted suspension (right).

In a second set of experiments adsorption to excipients was investigated. Therefore, methods to analyse the colour in the reconstituted suspension should be developed. To obtain a clear solution for measurement with colorimetry, two methods were evaluated.

3.4.2.4. Adsorption of the colourants onto excipients. When a reconstituted suspension containing erythrosine and quinoleine yellow, was passed through the filter, the following was observed (Fig. 5): a clear, yellow solution was obtained, which means that quinoleine yellow passes trough the filter but not erythrosine; on the paper filter some remains of quinoleine yellow, and a clear red colour, of course from the erythrosine, adsorbed on the non-water-soluble excipients, were noticed. Similarly, colour remains were noticed on other filters too and seemed therefore not suitable for our purpose.

Therefore, centrifugation was tried.

At least 12,000 rpm and 20 min centrifugation were required to obtain a clear solution from the reconstituted suspension for further investigation. Measurement of the absorbance of the solution on the first day confirmed the recovery of around 100% for quinoleine yellow and Sunset yellow. In contrast, as can be noticed for erythrosine, the colour is totally adsorbed to the precipitate, consisting of the non-water-soluble excipients in the suspension (Fig. 5). It is clear that erythrosine cannot be quantified as such.

In the following part, the adsorption of both colourants on the excipients of the suspension during 25 days was investigated. A mixture of quinoleine/erythrosine in a fixed ratio but with different amounts, and Sunset yellow, which concentration was selected from literature, were added to a colourless suspension.

The composition of the suspensions were:

- suspension 1: 0.80 mg/100 ml erythrosine and 20 mg/100 ml quinoleine;
- suspension 2: 0.56 mg/100 ml erythrosine and 14 mg/100 ml quinoleine;
- suspension 3: 0.32 mg/100 ml erythrosine and 8 mg/100 ml quinoleine;
- suspension 4: 10 mg/100 ml Sunset yellow.

The results are presented in Fig. 6.

Only very slight adsorption was noticed for quinoleine yellow. For Sunset yellow, no changes were observed during 25 days. In contrast, for erythrosine, important adsorption was noticed as the red colour was disappearing in suspensions 1 and 2. They are both turning into yellow coloured suspensions, as quinoleine yellow is still present.

In conclusion, Sunset yellow is selected. It did not absorb on the packaging, nor onto non-water-soluble excipients in the suspension.

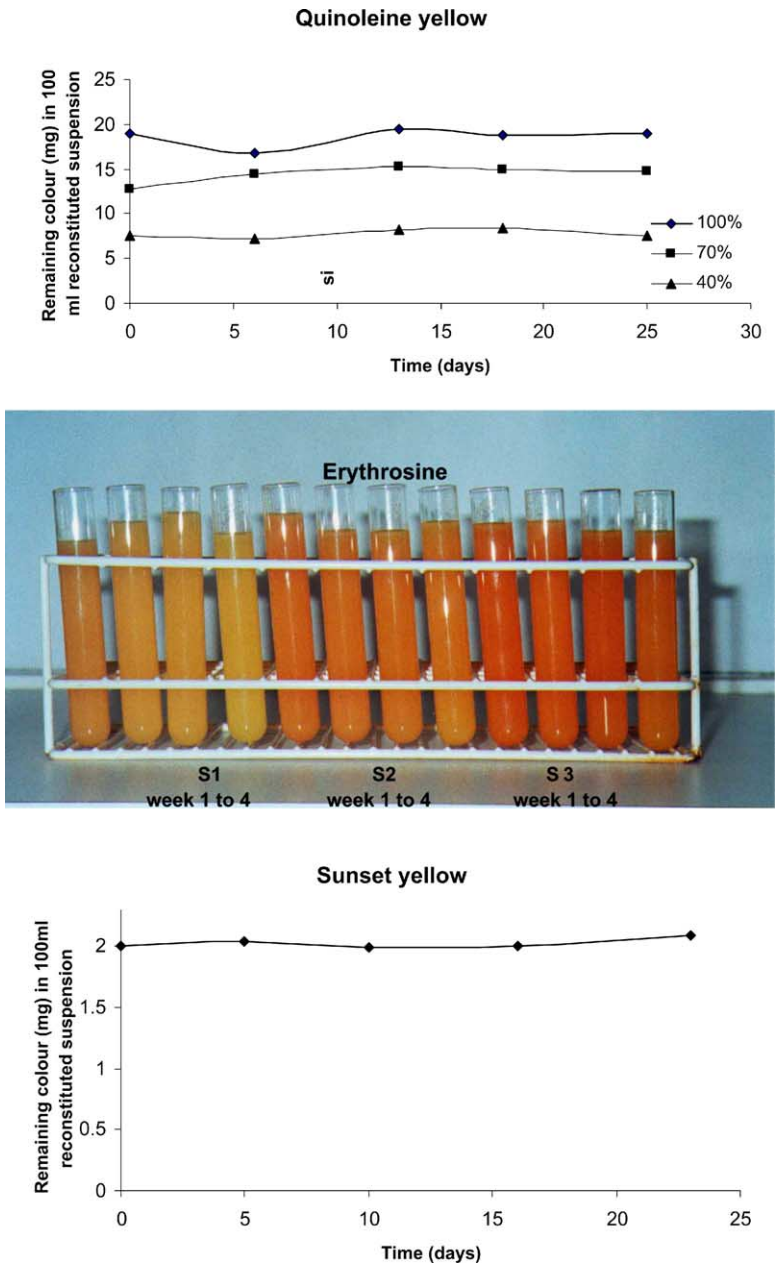

Fig. 6. Influence of the excipients on the stability of the colour in the reconstituted suspension: for quinoleine yellow; for erythrosine; for Sunset yellow.

4. General conclusion

In the present study, a powder for an oral suspension was developed. The concentration of the suspending agents, xanthan gum and Avicel® CL611 was optimised by aid of the Doehlert design, to obtain a permanently physical stability of the suspension after reconstitution. This characteristic is required due to

the low dosage of the drug in the suspension, namely 3 mg/ml. Viscosity was taken as response in the design. The most suitable amounts of the suspending agents, xanthan gum and Avicel® CL611 in the suspension were 0.20 and 2%; 0.20 and 2.4%; and, 0.20 and 2.3%, respectively for artesunate, artemether and dihydroartemisinin. An initial stability study showed sufficient chemical stability of the active, artemether and dihydroartemisinin, in the reconstituted suspension during the whole period of administration.

In contrast, artesunate showed degradation, despite enhancement of citric acid amount, which lowers the partial solubility of artesunate in the aqueous phase of the suspension.

For the artemether suspension, as well as the taste, as the colouration were optimised. Via taste tests with African children and Belgian students, sodium saccharinate and tutti-frutti were selected to mask the bitter taste of artemether. From the tests with the colourants, Sunset yellow was selected as it showed good stability in the reconstituted suspension and did not adsorb onto plastics and glass or other excipients in the suspension. Finally, the following composition was selected for a dry powder with artemether as active compound: artemether 300 mg, Avicel® CL611 2 g, xanthan gum 200 mg, crystalline saccharose 35 g, citric acid monohydrate 150 mg, Nipagine® 80 mg, Nipasol® 20 mg, sodium saccharinate 250 mg, tutti-frutti 250 mg and Sunset yellow 10 mg, reconstitutable to 100 ml in water.

The presented formulation seemed to be a good base for preparing low-dose suspension of the active compounds in the range of 1 to 10 mg/ml, in which the aestethic aspect of the suspension was optimised.

References

BASF fine chemicals, 1997. Generic Drug Formulations, 1st ed. BASF Aktiengesellschaft (Ludwichshafen, Germany).

Batty, K.T., Ilett, K.F., Davis, T., Davis, M.E., 1996. Chemical stability of artesunate injection and proposal for its administration by intravenous infusion. J. Pharm. Pharmacol. 48, 22–26.

Bhargava, H.N., Nicolai, D.W., 1989. Topical solutions. In: Lieberman, H.A., Rieger, M.M., Banker, G.S. (Eds.), Pharmaceutical Dosage Forms: Disperse Systems, vol. 2, 1st ed. Marcel Dekker Inc., New York, USA, pp. 265–316 (Chapter 7).

Bos, C.E., 1990. Optimisation of direct compression tablet formulations for use in tropical countries. In: Tropical Tablets: The Development of Tablet Formulations for Use in Tropical Countries. Doctoral Thesis, Rijksuniversiteit Groningen, pp. 127–140.

Harwood, R.J., Luber, J.R., Sunbery, E.W., 1989. Antacids and clay products. In: Lieberman, H.A., Rieger, M.M., Banker, G.S. (Eds.), Pharmaceutical Dosage Forms: Disperse Systems, vol. 2, 1st ed. Marcel Dekker Inc., New York, USA, pp. 205–230 (Chapter 5).

Hu, Y., Massart, D.L., 1989. Uniform shell designs for optimisation in reversed phase liquid chromatography. J. Chromatogr. 485, 311–323.

Karbwang, J., Na-Bangchang, K., Thanavibul, A., Molunto, P., 1998. Plasma concentrations of artemether and its major plasma metabolite, dihydroartemisinin, following a 5-day regimen of oral artemether in patients with uncomplicated Falciparum malaria. Ann. Trop. Med. Parasitol. 92, 31–36.

Looareesuwan, S., Wilairatana, P., 1998. The rational use of qinghaosu and its derivatives: what is the future of new compounds? Med. Trop. (Mars.) 58, 89–92.

Martin, A., Bustamante, P., Chun, A.H.C., 1993a. Part 17: Rheology. In: Physical Pharmacy, 4th ed. Lea & Febiger, Philadelphia, USA, pp. 453–476.

Martin, A., Bustamante, P., Chun, A.H.C., 1993b. Part 12: Kinetics. In: Physical Pharmacy, 4th ed. Lea & Febiger, Philadelphia, USA, pp. 284–323.

Martin, A., Bustamante, P., Chun, A.H.C., 1993c. Part 18: Coarse dispersions. In: Physical Pharmacy, 4th ed. Lea & Febiger, Philadelphia, USA, pp. 477–511.

Massart, D.L., Buydens, L.M.C., De Jong, S., Lewi, P.I., Smeyers-Verbeke, J., 1989. Uniform shell designs for optimisation in reversed phase liquid chromatography. J. Chromatogr. 485, 31–323.

Navaratnam, V., Mansor, S.M., Sit, N.W., Grace, J., Li, Q., Olliaro, P., 2000. Pharmacokinetics of artemisinin-type compounds. Clin. Pharmacokinet. 39, 255–270.

Ofner III, C.M., Schnaare, R.L., Schwartz, J.B., 1989. Oral aqueous suspensions. In: Lieberman, H.A., Rieger, M.M., Banker, G.S. (Eds.), Pharmaceutical Dosage Forms: Disperse Systems, vol. 2, 1st ed. Marcel Dekker Inc., New York, USA, pp. 231–264 (Chapter 6).

Plaizier-Vercammen, J.A., 1991. Preparation and rheological behaviour of a stable enema containing metaminosalicylic acid. Pharmakon 85, 13–19.

Vankeerbergen, P., Smeyers-Verbeke, J., Massart, D.L., 1996. Decision support system for run suitability checking and explorative method validation in electrothermal atomic absorption spectrophotometry. J. Anal. At. Spectr. 11, 149–158.

Worthington, J., 2000. Love at first bite: optimising flavour quality in pharmaceutical formulations. Pharmaceutic. Formulation Qual. 29–33.